



Hydrogel Substrates of Collagen-Starch-Selenium Complexes for Stimulating Plant Metabolism

Dirce S. Gomez-Galicia¹, Jesús A. Claudio-Rizo^{1*}, Denis A. Cabrera-Munguía¹, Maria I. León-Campos¹, Juan J. Mendoza-Villafaña¹, Tirso E. Flores-Guía¹ & Lucía F. Cano-Salazar¹

¹Department of Advanced Materials, Faculty of Chemical Sciences, Autonomous University of Coahuila, Blvd. Venustiano Carranza S/N, República, 25280 Saltillo, Coahuila, México. Email: jclaudio@uadec.edu.mx*



DOI: https://doi.org/10.38177/ajast.2024.8202

Copyright © 2024 Dirce S. Gomez-Galicia et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Article Received: 09 February 2024

Article Accepted: 19 April 2024

Article Published: 29 April 2024

ABSTRACT

Various selenium (IV) complexes with essential amino acids such as phenylalanine, histidine, and tryptophan were encapsulated within polymeric matrices in hydrogel state based on collagen-starch, generating the biomatrices Col-St(Se-F), Col-St(Se-H), and Col-St(Se-T), respectively. I mg of each type of selenium complex was employed per mass ratio in matrices consisting of 60% collagen and 18% starch by mass. The advanced materials were surface-characterized using scanning electron microscopy (SEM), and their properties such as swelling capacity and degradation rate in commercial vegetable substrate were also evaluated. The amino acid involved in the selenium complex forms characteristic granules that determine the microstructure and porosity of the biomatrix; reporting that in the case of all biomatrices with selenium complexes, swelling capacities exceeding 2900% are shown, resulting statistically significant compared to the sole collagen-starch matrix. Likewise, the matrices exhibit resistance to degradation in the presence of the commercial vegetable substrate for up to 30 days of evaluation. *In vitro* biocompatibility studies with green and red tomato cells derived from fresh seeds reveal that the biomatrices Col-Str(Se-F) and Col-Str(Se-H) overstimulate the metabolism of green tomato cells, while for red tomato cells, greater stimulation is appreciated in the Col-Str(Se-T) biomatrix. The chemical composition of the biomatrices promotes the proliferation of these types of plant cells as inspected by epifluorescent microscopy. Such advanced materials could be successfully applied in agricultural applications related to tomato cultivation by regulating water capacity, substrate biodegradation, and stimulated metabolism to promote tomato growth.

Keywords: Substrate; Hydrogel; Collagen; Starch; Amino acid; Selenium-complex; Biomatrix; Tomato; Agricultural applications; Biofertilizer.

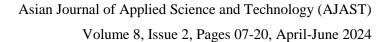
1. Introduction

Hydrogel substrates have emerged as a promising innovation in modern agriculture, offering a versatile solution for optimizing plant growth, particularly in crops like tomatoes [1]. These advanced materials, characterized by their high water retention capacity and ability to provide a controlled release of nutrients, are revolutionizing traditional farming practices [2]. In recent studies focusing on tomato cultivation, researchers have explored the application of hydrogel substrates to enhance plant growth and yield. By incorporating hydrogel-based matrices into the soil or potting mix, growers can effectively manage water availability, nutrient uptake, and root aeration, crucial factors for healthy tomato plants [1-2].

One of the key advantages of hydrogel substrates is their water retention capability. These materials can absorb and retain large quantities of water, reducing the frequency of irrigation and minimizing water wastage [1-2]. For tomato plants, maintaining optimal soil moisture levels is essential for preventing issues like blossom end rot and ensuring consistent growth throughout the growing season [3]. Furthermore, hydrogel substrates offer a controlled release of nutrients, delivering essential elements such as nitrogen, phosphorus, and potassium directly to the roots as needed [4]. This targeted nutrient delivery system promotes robust root development, vigorous foliage growth, and increased fruit production in tomato plants.

Moreover, hydrogel substrates provide excellent aeration to the root zone, preventing soil compaction and allowing for better oxygen uptake by the roots. This improved root environment encourages healthy root proliferation, enhances nutrient absorption efficiency, and ultimately leads to stronger, more resilient tomato plants [1-5]. In addition to their agronomic benefits, hydrogel substrates contribute to sustainable agriculture practices by reducing







water consumption, minimizing fertilizer runoff, and optimizing resource use efficiency [5]. By integrating these innovative materials into tomato cultivation systems, farmers can improve crop productivity while minimizing environmental impact.

Collagen-starch hydrogels have emerged as a revolutionary tool in modern plant cultivation, offering a sustainable and efficient method to optimize growth conditions and enhance crop yields. These innovative materials, derived from a combination of collagen and starch, possess unique properties that make them ideal for controlling various aspects of plant growth [6]. One of the key advantages of collagen-starch hydrogels is their exceptional water retention capacity [6]. These hydrogels can absorb and retain significant amounts of water, effectively buffering moisture levels in the soil or growing medium [7]. By maintaining consistent soil moisture, collagen-starch hydrogels help prevent both underwatering and overwatering, providing plants with the optimal hydration they need for healthy growth. Besides, collagen-starch hydrogels act as reservoirs for essential nutrients, gradually releasing them into the surrounding soil or substrate as needed. This controlled nutrient release mechanism ensures that plants receive a steady supply of vital elements such as nitrogen, phosphorus, potassium and other microelements, promoting robust growth and development throughout the growing season [7-8].

In addition to their water retention and nutrient delivery capabilities, collagen-starch hydrogels also improve soil structure and aeration [8]. These materials help loosen compacted soils, allowing for better root penetration and oxygen uptake. Enhanced soil aeration stimulates root growth and facilitates nutrient absorption, ultimately leading to stronger, more resilient plants. Collagen-starch hydrogels can be easily incorporated into various cultivation systems, including traditional soil-based methods and hydroponic or aquaponic setups [9]. Whether used in greenhouse production, urban farming initiatives, or large-scale agriculture, these hydrogels offer a versatile solution for optimizing plant growth and maximizing yields [10]. Otherwise, the use of collagen-starch hydrogels aligns with sustainable farming practices by reducing water consumption, minimizing nutrient leaching, and promoting resource efficiency. By enhancing the water and nutrient-holding capacity of soils and substrates, these hydrogels help minimize environmental impact while improving overall crop performance.

The utilization of selenium biocomplexes presents a novel approach to regulating the growth of tomato plants, offering a sophisticated means to enhance their development and productivity [11]. These biocomplexes, derived from selenium compounds integrated with essential amino acids such as phenylalanine, histidine, and tryptophan, could exert a multifaceted influence on the physiological processes of tomato plants.

One of the primary benefits of selenium biocomplexes lies in their capacity to modulate plant metabolism. Selenium, as an essential micronutrient, plays a crucial role in various biochemical pathways, including antioxidant defense mechanisms and hormone regulation [12]. By incorporating selenium biocomplexes into the growth regimen of tomato plants, growers can effectively optimize metabolic activities, leading to improved nutrient uptake, enhanced stress tolerance, and enhanced overall growth. Also, selenium biocomplexes contribute to the promotion of plant resilience and vigor [12]. These compounds bolster the plant's natural defense mechanisms against environmental stresses such as drought, salinity, and disease [12-13]. By fortifying the plant's antioxidant systems and strengthening cell walls, selenium biocomplexes help mitigate the adverse effects of stressors, ensuring





continued growth and productivity even under challenging conditions [12-13]. Besides, selenium biocomplexes play a pivotal role in enhancing fruit quality and yield in tomato plants. Selenium is known to positively influence fruit development, contributing to increased fruit size, enhanced flavor, and extended shelf life [13]. By incorporating selenium biocomplexes into the cultivation process, growers can expect to harvest tomatoes of superior quality, with enhanced nutritional value and market appeal. In addition to their physiological effects, selenium biocomplexes contribute to sustainable agricultural practices by minimizing environmental impacts. Selenium, when applied in biocomplex form, exhibits high bioavailability and targeted uptake by plants, reducing the need for excessive fertilizer application and minimizing nutrient runoff [13]. This targeted nutrient delivery system promotes resource efficiency and minimizes environmental pollution, aligning with the principles of sustainable agriculture [12-13].

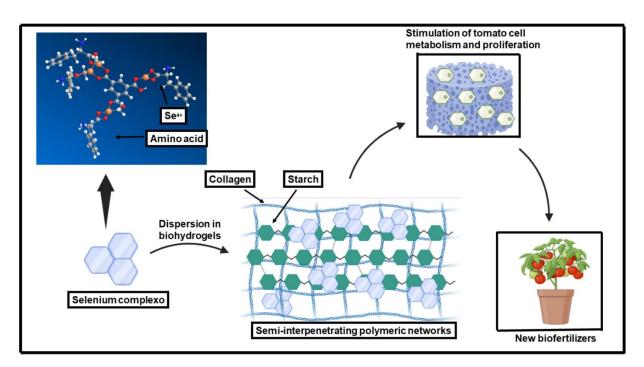
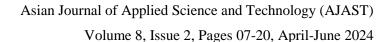


Figure 1. Preparation of hydrogel substrate based on collagen-starch and selenium biocomplexes for potential application in the cultivation of tomato plants

Based on these background findings, the present study aims to prepare hydrogel substrates composed of collagen-starch supplemented with different selenium bioactive complexes for potential application in tomato plant growth. The working hypothesis proposes that the chemical structure of the amino acid forming the selenium biocomplex (phenylalanine, histidine, or tryptophan) will have an effect on the structural, physicochemical, and *in vitro* biological performance properties using red and green tomato cells (Figure 1). This research represents a novel approach to optimizing plant growth through the strategic incorporation of selenium bioactive complexes into hydrogel substrates. By investigating how the choice of amino acid within the selenium complex influences substrate properties and biological activity, this study aims to contribute valuable insights into the development of tailored hydrogel formulations for improved tomato cultivation practices. Through a combination of experimental methodologies including synthesis, characterization, and biological assays, this study seeks to elucidate the relationship between selenium biocomplex composition and substrate performance. By systematically analyzing





the structural and functional properties of the prepared hydrogels and assessing their impact on tomato cell cultures, this research aims to provide a comprehensive understanding of the potential benefits and limitations of selenium-enhanced hydrogel substrates for agricultural applications. Ultimately, the findings of this study have the potential to inform the development of innovative hydrogel-based solutions for sustainable and efficient tomato production. By harnessing the unique properties of collagen-starch hydrogels supplemented with selenium bioactive complexes, growers may be able to optimize nutrient delivery, promote plant health, and enhance overall crop yield, contributing to the advancement of agricultural practices and food security initiatives.

1.1. Study Objectives

The following are the main objectives of this study. (i) Synthesize collagen-starch-selenium complexes biomatrices with potential application in plant growth. (ii) Analyze the surface microstructure of the biomatrices. (iii) Evaluate the maximum swelling capacity of the biomatrices. (iv) Study degradation profiles on substrate for commercial vegetable materials. (v) Investigate the effect of the chemical composition of the biomatrices on the metabolic activity of red and green tomato cells using the MTT assay. (vi) Determine the proliferation of red and green tomato cells using epifluorescent staining assays.

2. Materials and Methods

In our scientific pursuit, porcine tendons became the cornerstone of our study, sourced diligently from the bustling local market. With meticulous care, we subjected these tendons to a series of experiments aimed at extracting type I collagen with specific molecular weights of 110 kDa and 220 kDa [14]. To embark on this journey, we gathered a selection of essential chemicals from the esteemed CTR Scientific. Sodium chloride, potassium chloride, monopotassium phosphate, sodium acid phosphate, and sodium hydroxide were among the foundational components. Additionally, 2-propanol, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), glycerol ethoxylate (GE), hexamethylenediisocyanate (HDI), trimesic acid, and a myriad of amino acids such as L-histidine, L-phenylalanine, and L-tryptophan were meticulously sourced. Furthermore, vital reagents including selenium (IV) chloride (SeCl₄), trimesic acid (TA), fluorescein and starch with a molecular weight of 500 KDa were procured from the reliable shelves of Sigma-Aldrich. Our endeavors in plant cell culture were bolstered by the acquisition of Murashige and Skoog (MS) culture media, also from Sigma-Aldrich. As our research extended beyond the confines of the laboratory, we sought the enriching embrace of nature itself. Vibrant tomato plants and seeds, teeming with life, were carefully acquired from a local greenhouse, ready to be seamlessly integrated into our scientific exploration. With each element meticulously assembled, our study poised at the intersection of meticulous experimentation and the vibrant essence of the natural world, ready to unravel new frontiers in scientific inquiry.

2.1. Synthesis of Biomaterial Matrices

In the pursuit of pioneering biomaterials, hydrogel matrices were meticulously crafted through a sequence of precisely orchestrated steps. Initially, Type I collagen, derived from porcine tendons via enzymatic hydrolysis, served as the foundational material. This collagen, characterized by molecular weights of 220,000 Da and 110,000 Da, underwent a method meticulously outlined in existing literature [14], ensuring the integrity of the resulting





biomatrix. Concurrently, the polyurethane crosslinker, an indispensable component of our matrices, was synthesized from glycerol ethoxylate (GE) and hexamethylenediisocyanate (HDI). The synthesis procedure, extensively documented elsewhere [15], yielded a crosslinker within the molecular weight range of 3000–7500 Da, thereby ensuring optimal structural integrity for the matrices. The centerpiece of our innovation, the selenium biocomplexes, were synthesized via hydrothermal methods. By utilizing SeCl₄ alongside amino acids L-tryptophan, L-histidine, and L-phenylalanine, along with trimesic acid (TA) as a co-ligand, a family of selenium biocomplexes, Se-F, Se-H and Se-T—were synthesized. Employing equimolar ratios of selenium and ligands, with a 30:60 ratio for TA:amino acid, hydrothermal conditions at 100 °C for 24 hours facilitated the formation of robust coordination polymer networks. Following synthesis, the resulting solid was meticulously filtered, washed with distilled water, and dried at 60 °C for 24 hours [16].

In the subsequent stages of matrix production, dispersions of each selenium biocomplex were incorporated into a collagen solution at a ratio of 1 mg of biocomplex using 24-well culture plates. Augmenting this suspension, 15 wt.% of polyurethane served as the crosslinking agent, reinforcing the structural integrity of the matrices. To further strengthen the matrix, starch, comprising 18-25 wt.%, was introduced into the mixture, resulting in the formation of semi-interpenetrating polymer network (semi-IPN) systems. Maintaining a pH of 7.4 with a phosphate-buffered solution (PBS 10X), the reaction mixture underwent incubation at 37°C for 24 hours, allowing for the maturation of the matrices. Table 1 provides insight into the chemical compositions and designations of the synthesized matrices, showcasing the culmination of meticulous design and precise execution in the realm of biomaterial innovation.

Table 1. Formulations and designations of hydrogel substrates indicating the percentage composition by mass

Hydrogel substrate	Collagen content (wt.%)	Starch content (wt.%)	Crosslinker content (wt.%)	Selenium biocomplex content (wt.%)
Col-St	60	25	15	0
Col-St(Se-F)	60	18	15	7
Col-St(Se-H)	60	18	15	7
Col-St(Se-T)	60	18	15	7

^{*}Note: Mass ratios (wt.%) are calculated by adding each mass for each chemical component.

2.2. Methods for Analyzing the Physical and Chemical Properties of Biomatrices

The surface microstructure analysis of the hydrogel substrates was conducted using a Jeol JSM 7600F scanning electron microscope (SEM), which provided detailed images. The maximum swelling capacity of the substrates was assessed by comparing the mass of the hydrogel state with that of the water-free state. This calculation offered valuable insights into the biomatrix's capability to absorb and retain water, which is essential for its effectiveness in a wide range of applications such as agricultural. To evaluate substrate degradation, experiments were conducted to measure mass loss under agricultural conditions, utilizing a commercial substrate for vegetables. All tests were carried out at room temperature to simulate real-world scenarios accurately.





2.3. Biocompatibility Tests Conducted with Tomato Plant Cells in a Controlled Laboratory Setting (in vitro)

In this study, cells obtained from both red and green tomato seeds were utilized. Initially, 500 mg of seeds were gathered and subjected to washing in a 2% ethanol solution for 30 minutes to eliminate any impurities. Following this, the seeds underwent individual homogenization for 15 minutes and subsequent centrifugation at 3000 rpm for 15 minutes to obtain plant cell pellets. These pellets were then seeded into culture dishes containing 30 mL of Murashige and Skoog (MS) medium and placed in an incubator at 25°C for 48 hours to promote proliferation and growth. The resulting cell cultures, originating from the seeds with an initial density of 100,000 cells/mL, were observed under a bright-field microscope, and cell counts were conducted using a Neubauer Chamber. To assess the metabolic activity of the cells growing on substrates, the tetrazolium salt reduction (MTT) assay was employed. This assay provides a means to quantify cellular viability and proliferation by measuring the conversion of MTT into formazan crystals, indicating active mitochondrial function. These *in vitro* experiments were aimed at assessing the compatibility of tomato plant cells with the substrates, offering insights into their potential suitability for various biomedical applications. Cell proliferation was assessed through fluorescent staining using a fluorescein cell staining agent and observed under a VELAB VE-146YT epifluorescence microscope.

The experiments were meticulously performed in triplicate to ensure the robustness and reliability of the data. Subsequently, the means and standard deviations were calculated for each dataset, offering a comprehensive understanding of the experimental outcomes. To examine the variance between the means of different experimental conditions, a One-way Analysis of Variance (ANOVA) was utilized. Subsequent to this analysis, the statistical significance was determined through a Tukey test, employing a confidence limit of 95% (*p<0.05*). This rigorous methodology guaranteed precise evaluation and comparison of the experimental results, enabling confident conclusions to be drawn from the research findings.

3. Results and Discussion

The surface microstructure of the hydrogel substrates was inspected by SEM, with micrographs for each substrate type presented in Figure 2a. The material without selenium complex (Col-St) exhibits a granular relief composed of interconnected collagen fibrils with characteristic porosity, with starch aggregates occluded within this matrix, demonstrating the successful generation of a semi-interpenetrating polymer network.

The chemical structure of the selenium biocomplex determines the formation of different topological changes in the collagen-starch matrix. In the case of Col-St(Se-F), a decrease in surface granulation with increased porosity of this substrate is observed; the hydrophobic regions of the aromatic ring of phenylalanine promote greater interaction with collagen fibrils, resulting in a more homogeneous surface. For Col-St(Se-H), clusters of different sizes are observed both occluded and exposed on the surface, and this type of result in this microstructure is associated with interactions of the imidazole ring that forms histidine, promoting both hydrophobic and hydrophilic interactions, thus forming these clusters when interacting with biopolymer chains. Finally, in the case of the substrate based on the selenium-tryptophan complex (Col-St(Se-T)), the generation of smaller clusters compared to Col-St(Se-H), mostly occluded on the surface, is perceived, and an alteration of the porosity is also appreciated; this can be attributed to the structure of the indole ring of tryptophan, which may promote greater hydrophilic interactions with





starch, decreasing the size of the granules generated superficially. Such changes in the morphology of the surface of these substrates can influence the vital functions of plant cells, stimulating or decreasing their metabolism due to alterations in the flow of nutrients and waste, as well as promoting cell adhesion that allows for the growth and proliferation of plant tissue [17].

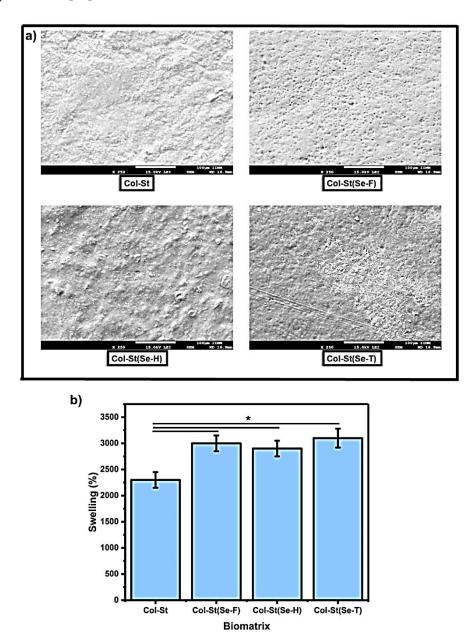


Figure 2. (a) Microstructural characteristics, and (b) swelling capacity of substrates in the collagen-starch hydrogel state reinforced with selenium biocomplexes

The swelling capacity shows a direct relationship with the type of surface microstructure that each substrate possesses. Swelling capacities of $2250 \pm 185\%$, $3000 \pm 210\%$, $2920 \pm 113\%$, and $3120 \pm 253\%$ are determined for Col-St, Col-St(Se-F), Col-St(Se-H), and Col-St(Se-T), respectively. Statistically significant differences are recorded when comparing swelling capacities that include selenium biocomplexes with respect to the pure Col-St matrix. It can be observed that increasing the grain size generated by the interaction with the amino acid that forms the selenium complex affects the water absorption capacity of the substrate. That is to say, the substrate



Col-St(Se-H), which promotes a larger grain size, shows a lower water absorption capacity, indicating that the granules have a repulsion effect with water molecules, reducing their ability to retain them in their swollen state. Similarly, the reduction in grain size for systems with Se-F and Se-T promotes greater water uptake, indicating that the porosity present in these substrates benefits water absorption into the semi-IPN matrix. Super-swelling capacities are reported for the substrates under study, indicating that they could modulate the supply of water to plant cells to promote germination and plant growth [10-11], representing hydrogel state substrates with potential applications in agriculture. Hydrogel state substrates that exhibit controlled water diffusion are excellent materials for promoting plant growth. Plant cells thrive in an extracellular matrix that mimics native cellulose, facilitating their proliferation. Furthermore, this modulation of water is crucial for generating the various cellular phenotypes that make up the plant, ultimately benefiting fruit formation and multiplication [18].

The degradation of substrates in hydrogel state was evaluated in the presence of commercial vegetable substrates, and the mass variation profiles are shown in Figure 3. From the second day of contact with this condition, all polymeric matrices reveal a mass loss ranging from 98%, which is associated with dehydration processes (removal of water from the substrate structure) due to contact with adsorbents forming the substrate such as minerals, sands, and natural fibers.

Regardless of the chemical nature of the selenium biocomplex, all substrates exhibit this trend. Residual masses after days of substrate study, fluctuating around 2%, indicate that the collagen-starch polymeric matrix formed by each type of selenium complex suggests that the matrices could reabsorb water if supplied and could keep both the substrate and seeds hydrated to initiate the germination process, taking advantage of the superabsorbent capacity that these substrates exhibit, representing a substrate system for plant growth with hydrological control, which is important nowadays in the search for materials for sustainable agriculture.

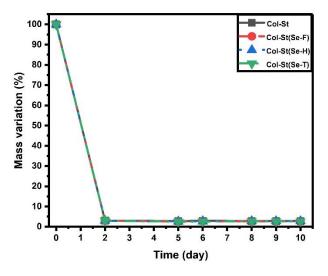


Figure 3. Mass variation profiles of substrates for agricultural application in the presence of commercial substrate for vegetables

The *in vitro* biological evaluation of collagen-starch-selenium complex hydrogel substrates involved assays of metabolic activity and proliferation detection using green and red tomato cells derived from seeds for each species. Regarding the results of metabolic activity (Figure 4a), it is observed for green tomato cells that there is a metabolic



stimulation when these cells grow in contact with substrates containing selenium complexes. Significant differences are found when comparing these results both at 24 h and 48 h compared to the control (PBS 1X) and the matrix without selenium complex (Col-St). The greatest metabolic stimulation was determined for green tomato cells growing on Col-St(Se-F) and Col-St(Se-H), indicating that the degradation products of these substrates enhance the metabolic activity of this type of cells. The influence of selenium on the growth of green tomatoes has been the subject of considerable research and experimentation in agricultural science. Selenium, an essential micronutrient for plants and animals, plays a crucial role in various physiological processes, including growth regulation, stress response, and antioxidant defense mechanisms [12-13].

Studies have shown that selenium supplementation can positively impact the growth and development of green tomato plants. Selenium functions as a cofactor for certain enzymes involved in photosynthesis and nitrogen metabolism, thereby enhancing the plant's ability to utilize sunlight and nutrients efficiently [11-13].

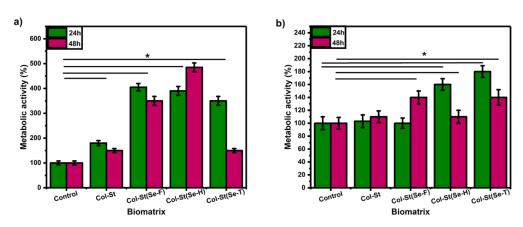


Figure 4. Evaluation of metabolic activity by the MTT salt reduction assay: (a) green tomato cells, and (b) red tomato cells

Moreover, selenium is known to enhance the plant's tolerance to various abiotic stresses such as drought, salinity, and heavy metal toxicity. By mitigating the harmful effects of environmental stressors, selenium supplementation can promote healthier and more vigorous growth in green tomato plants [11-13]. Besides, selenium is involved in the synthesis of phytochemicals and secondary metabolites in plants, including antioxidants such as vitamin C and flavonoids [11-13]. These compounds contribute to the overall health and nutritional quality of green tomatoes, making them more resilient to diseases and pests while also enhancing their flavor and nutritional value. However, it's essential to note that selenium supplementation must be carefully managed to avoid toxicity issues, as excessive selenium levels can have adverse effects on plant growth and development [19]. Proper dosing and application methods are crucial to harnessing the beneficial effects of selenium on green tomato growth while minimizing the risk of toxicity.

Regarding the metabolism of cells derived from red tomatoes (Figure 4b), a lesser stimulation of their metabolism compared to green tomato cells is observed. However, statistically significant differences are determined in substrates with selenium complexes compared to the control and the Col-St matrix. In this case, substrates Col-St(Se-H) and Col-St(Se-T) show metabolic stimulation at 24 hours of contact with these substrates, while at 48 hours, the substrate Col-St(Se-F) shows an increase in metabolic stimulation. In both cases under study, no



cytotoxic effects are observed that would hinder the growth and fundamental functions of both types of tomato cells. Regarding the microstructure evaluated by SEM for all substrates under study, it is revealed that in the case of green tomato cells, metabolic stimulation is related to surfaces that generate larger granules occluded in the collagen-starch polymeric matrix, while for red tomato cells, there is greater metabolic stimulation due to the presence of smaller molecular size occluded granules. These granules are associated with the release of components that cells use for their metabolic functions, such as selenium, the amino acids phenylalanine, histidine, or tryptophan, depending on the composition, representing novel formulations of biofertilizers that effectively stimulate the metabolism of tomato cells. This could promote their growth in shorter periods of time and avoid the use of traditional fertilizers.

The proliferation capacity of both green and red tomato cells was inspected using epifluorescent staining with fluorescein as a vital membrane marker. Fluorescein binds to the proteins of the plant cell wall, allowing the visualization of confluent active cells (with active metabolism). These fluorescent micrographs are presented in Figure 5. For green tomato cells (Figure 5a), dense populations of cells growing on the Col-St, Col-St(Se-F), and Col-St(Se-H) bio-substrates are observed, while a smaller cell population was observed on Col-St(Se-T). For red tomato cells (Figure 5b), wide densities of proliferating cells are observed in all substrates under study, regardless of the substrate composition tested. These results are consistent with the findings from the MTT metabolic activity assays.

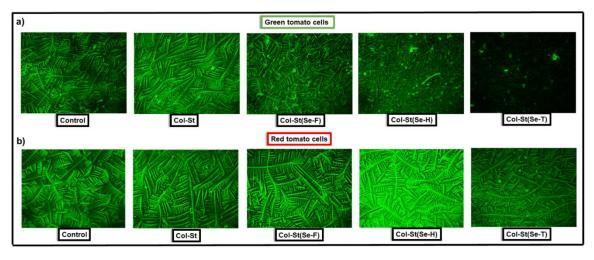
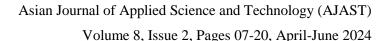


Figure 5. Epifluorescent proliferation of plant cells stained with fluorescein: (a) cells derived from green tomato, and (b) cells derived from red tomato

The cell proliferation of tomato cells is highly facilitated by having a super-stimulated metabolic activity due to the chemical composition of the substrates containing selenium complexes. This indicates a controlled dosage of nutrients towards the cell, allowing them to thrive and proliferate. Phenylalanine can positively influence tomato growth by regulating various physiological processes, including root development, stress response, flowering, fruit set, and nutrient transport. However, it's essential to consider the balance of phenylalanine with other nutrients and environmental factors to optimize its beneficial effects on tomato plants [20]. By the other hand, histidine plays diverse and essential roles in tomato growth and development, including protein synthesis, metal transport, stress response, flowering, fruit development, and nitrogen metabolism. Maintaining adequate histidine levels and





balance in the plant's physiology is crucial for maximizing tomato yield and quality [21]. Finally, tryptophan plays multifaceted roles in tomato growth and development, including its involvement in hormone regulation, root development, stress response, flowering, fruit development, and secondary metabolite production. Optimizing tryptophan levels and metabolism in tomato plants can contribute to enhanced growth, productivity, and resilience in various environmental conditions [22].

4. Conclusion

In conclusion, various selenium (IV) complexes with essential amino acids, namely phenylalanine, histidine, and tryptophan, were successfully encapsulated within polymeric matrices in a hydrogel state based on collagen-starch, resulting in the formation of biomatrices denoted as Col-St(Se-F), Col-St(Se-H), and Col-St(Se-T), respectively. Each type of selenium complex (7%) was incorporated at a mass ratio of 1 mg per 60% collagen and 18% starch by mass. The surface characteristics of these advanced materials were analysed using scanning electron microscopy (SEM), revealing distinct microstructures influenced by the amino acid involved in the selenium complex. Furthermore, the biomatrices exhibited exceptional swelling capacities exceeding 2900%, statistically significant compared to matrices without selenium complexes. Moreover, they displayed remarkable resistance to degradation in the presence of commercial vegetable substrates for up to 30 days, showcasing their potential longevity in agricultural applications. In vitro biocompatibility studies conducted with green and red tomato cells derived from fresh seeds demonstrated that Col-St(Se-F) and Col-St(Se-H) biomatrices exhibited enhanced metabolic stimulation in green tomato cells, while Col-St(Se-T) showed greater stimulation in red tomato cells. This observation was further supported by epifluorescent microscopy, which revealed increased cell proliferation promoted by the chemical composition of the biomatrices. These findings suggest that these advanced materials hold promise for agricultural applications, particularly in tomato cultivation. By regulating water capacity, substrate biodegradation, and stimulating metabolism, they have the potential to significantly enhance tomato growth and yield. Overall, the development of such biomatrices represents a novel approach towards sustainable agricultural practices aimed at optimizing plant growth and productivity.

5. Future suggestions

The following are some future suggestions. (i) Study the germination process of red and green tomato seeds using commercial vegetable substrate. (ii) Evaluate the growth characteristics of the plants generated by the use of these biomatrices. (iii) Determine the water distribution efficiency of the biomatrices in tomato crops under controlled conditions such as greenhouses. (iv) Utilize local soils to induce the growth of red and green tomatoes using these biomatrices. (v) Assess the environmental and sustainable safety of these biomatrices as potential biofertilizers for tomato crops.

Declarations

Source of Funding

This research work is financially supported by Consejo Nacional de Humanidades, Ciencia y Tecnología (CONAHCyT) under the FORDECYT-PRONACES-6660 and CF-2023-G-1348 projects.





Competing Interests Statement

The authors have declared that no competing financial, professional or personal interests exist.

Consent for Publication

All the authors contributed to the manuscript and consented to the publication of this research work.

Authors' Contributions

All the authors took part in literature review, research, and manuscript writing equally.

Availability of data and material

Supplementary information is available from the authors upon reasonable request.

References

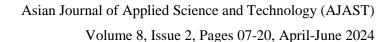
- [1] Wu, Y., Li, S., & Chen, G. (2023). Hydrogels as water and nutrient reservoirs in agricultural soil: a comprehensive review of classification, performance, and economic advantages. Environ Dev Sustain. https://doi.org/10.1007/s10668-023-03706-y.
- [2] Palanivelu, S.D., Armir, N.A.Z., Zulkifli, A., Hair, A.H.A., & Salleh, K.M. (2022). Hydrogel Application in Urban Farming: Potentials and Limitations-A Review. Polymers, 14(13): 2590. https://doi.org/10.3390/polym14132590.
- [3] Madramootoo, C.A., Jain, A., Oliva, C., Wang, Y., & Abbasi, N.A. (2023). Growth and yield of tomato on soil amended with waste paper based hydrogels. Sci Hortic., 310: 111752. https://doi.org/10.1016/j.scienta. 2022.111752.
- [4] Hu, Z.Y., Chen, G., Yi, S.H., Wang, Y., Liu, Q., & Wang, R. (2021). Multifunctional porous hydrogel with nutrient controlled-release and excellent biodegradation. J Environ Chem Eng., 9(5): 106146. https://doi.org/10.1016/j.jece.2021.106146.
- [5] Patra, S.K., Poddar, R., Brestic, M., Acharjee, P.U., Bhattacharya, P., Sengupta, S., Pal, P., Bam, N., Biswas, B., Barek, V., Ondrisik, P., Skalicky, M., & Hossain, A. (2022). Prospects of Hydrogels in Agriculture for Enhancing Crop and Water Productivity under Water Deficit Condition. Int J Polym Sci., 2022: 4914836. https://doi.org/10.1155/2022/4914836.
- [6] Caldera-Villalobos, M., Claudio-Rizo, J.A., Rodríguez-Estrada, V.A., Cabrera Munguía, D.A., & Becerra-Rodríguez, J.J. (2023). Effect of the content of starch on the biocompatibility, bacterial inhibition, and drug release performance of semi-IPN collagen-polyurethane hydrogels. J Macromol Sci A., 60(2): 124–134. https://doi.org/10.1080/10601325.2023.2166842.
- [7] Tariq, Z., & Iqbal, D.N. (2023). Significance of biopolymer-based hydrogels and their applications in agriculture: a review in perspective of synthesis and their degree of swelling for water holding. RSC Adv., 13(35): 24731–24754. https://doi.org/10.1039/D3RA03472K.





- [8] Adjuik, T.A., Nokes, S.E., Montross, M.D., & Wendroth, O. (2022). The Impacts of Bio-Based and Synthetic Hydrogels on Soil Hydraulic Properties: A Review. Polymers, 14(21): 4721. https://doi.org/10.3390/polym14214721.
- [9] Ma, L., Chai, C., Wu, W., Qi, P., Liu, X., & Hao, J. (2023). Hydrogels as the plant culture substrates: A review. Carbohydr Polym., 305: 120544. https://doi.org/10.1016/j.carbpol.2023.120544.
- [10] Azeem, M.K., Islam, A., Khan, R.U., Rasool, A., Qureshi, M.A.u.R., Rizwan, M., Sher, F., & Rasheed, T. (2023). Eco-friendly three-dimensional hydrogels for sustainable agricultural applications: Current and future scenarios. Polym Advan Technol., 34(9): 3046–3062. https://doi.org/10.1002/pat.6122.
- [11] Schiavon, M., & Pilon-Smits, E.A.H. (2017). The fascinating facets of plant selenium accumulation—biochemistry, physiology, evolution and ecology. New Phytol., 213(4): 1582–1596. https://doi.org/10.1111/nph. 14378.
- [12] Liu, H., Xiao, C., Qiu, T., Deng, J., Cheng, H., Cong, X., Cheng, S., Rao, S., & Zhang, Y. (2023). Selenium Regulates Antioxidant, Photosynthesis, and Cell Permeability in Plants under Various Abiotic Stresses: A Review. Plants, 12(1): 44. https://doi.org/10.3390/plants12010044.
- [13] Puccinelli, M., Malorgio, F., & Terry, L.A. (2019). Effect of selenium enrichment on metabolism of tomato (*Solanum lycopersicum*) fruit during postharvest ripening. J Sci Food Agric., 99(5): 2463–2472. https://doi.org/10. 1002/jsfa.9455.
- [14] Claudio-Rizo, J.A., Rangel-Argote, M., Castellano, L.E., Delgado, J., Mata-Mata, J.L., & Mendoza-Novelo, B. (2017). Influence of residual composition on the structure and properties of extracellular matrix derived hydrogels. Mater Sci Eng C Mater Biol Appl., 79: 793–801. https://doi.org/10.1016/j.msec.2017.05.118.
- [15] Caldera-Villalobos, M., Ramos-Montañez, D.G., Cabrera-Munguía, D.A., Becerra-Rodriguez, J.J., Rodríguez-Fuentes, N., & Claudio-Rizo, J.A. (2023). Biocompatible hydrogels comprised of collagen, chitosan and polyurethane with potential applications for wound healing and controlled drug release. Polym Int., 2023: 6590. https://doi.org/10.1002/pi.6590.
- [16] Caldera-Villalobos, M., Cabrera-Munguía, D.A., Becerra-Rodríguez, J.J., & Claudio-Rizo, J.A. (2022). Tailoring biocompatibility of composite scaffolds of collagen/guar gum with metal—organic frameworks. RSC Adv., 12(6): 3672–3686. https://doi.org/10.1039/D1RA08824F.
- [17] Szatanik-Kloc, A., Horn, R., Lipiec, J., Siczek, A., & Boguta, P. (2019). Initial growth and root surface properties of dicotyledonous plants in structurally intact field soil and compacted headland soil. Soil Tillage Res., 195: 104387. https://doi.org/10.1016/j.still.2019.104387.
- [18] Poorna, M.R., Jayakumar, R., Chen, J.P., & Mony, U. (2021). Hydrogels: A potential platform for induced pluripotent stem cell culture and differentiation. Colloid Surfaces B., 207: 111991. https://doi.org/10.1016/j.colsurfb.2021.111991.
- [19] Hasanuzzaman, M., & Bhuyan, M. (2020). Selenium Toxicity in Plants and Environment: Biogeochemistry and Remediation Possibilities. Plants, 9(12): 1711. https://doi.org/10.3390/plants9121711.

OPEN ACCESS





[20] Chang, A., Lim, M.H., Lee, S.W., Robb, E.J., & Nazar, R.N. (2008). Tomato phenylalanine ammonia-lyase gene family, highly redundant but strongly underutilized. J Biol Chem., 283(48): 33591–601. https://doi.org/10.1074%2Fjbc.M804428200.

[21] Picton, S., Gray, J.E., Payton, S., Barton, S.L., Lowe, A., & Grierson, D. (1993). A histidine decarboxylase-like mRNA is involved in tomato fruit ripening. Plant Mol Biol., 23(3): 627–31. https://doi.org/10. 1007/bf00019310.

[22] Yang, L., Ma, Y., & Ye, J. (2021). In vivo detection of L-tryptophan in tomatoes using multi-walled carbon nanotubes and poly (sulfosalicylic acid) film modified graphite rod electrode. Biosens Bioelectron., 9: 100086. https://doi.org/10.1016/j.biosx.2021.100086.

